Anti-hyperglycemic Effects of *Vaccinium arctostaphylos* L. Fruit and Leaf Extracts in Alloxan-Induced Diabetic Rats

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Abstract

Background: ***Vaccinium arctostaphylos*** **L. (Caucasian whortleberry) fruit is used as an anti-hyperglycemic agent for treatment of diabetes mellitus.**

Objective: The effects of whortleberry fruit and leaf extracts on the blood levels of fasting glucose, HbA1c (glycosylated hemoglobin), insulin, creatinine and liver enzymes SGOT and SGPT in alloxan-diabetic rats as well as LD_{50}s of the extracts in rats were studied.

Methods: The effects of 2 months daily gavage of each extract at the doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg on the parameters after single alloxan intraperitoneal injection at a dose of 125 mg/kg in the rats were evaluated. To calculate LD_{50} (median lethal dose), each extract was gavaged to groups of 30 healthy male and female Wistar rats at various doses once and the number of dead animals in each group within 72 hours was determined.

Results: Alloxan injection resulted in significant increase of fasting glucose and HbA1c levels but decreased insulin levels significantly. Oral administration of whortleberry fruit and leaf extracts (each at the doses of 250, 500 and 1000 mg/kg) significantly reduced the fasting glucose and HbA1c levels but significantly increased the insulin levels without any significant effects on the SGOT, SGPT and creatinine levels in the diabetic rats compared with the control diabetic rats. The LD_{50}s of the extracts were more than 15 g/kg.

Conclusion: Whortleberry fruits and leaves may have anti-hyperglycemic and blood insulin level elevating effects without hepatic and renal toxicities in the alloxan-diabetic rats and are relatively nontoxic in rats.

Keywords: Diabetes mellitus, Rat, ***Vaccinium arctostaphylos*** **L.**

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Introduction

Diabetes mellitus is the most prevalent metabolic disorder. The prevalence of diabetes is nearly 6% of the population and the diabetes type 2 constitutes 90 to 95% of it. If diabetes is not duly treated, it will lead to serious complications such as atherosclerosis, retinopathy, nephropathy, neuropathy and limbs gangrene. The complications are the main causes of morbidities and mortalities due to diabetes [1, 2]. While insulin and oral anti-hyperglycemic drugs such as biguanides, sulfonylureas, thiazolidinediones and alpha-glucosidase inhibitors are the cornerstone of the diabetes treatment, they have important adverse effects and cannot always maintain euglycemia and prevent diabetes complications significantly [3, 4]. Thus there is a continuing need for alternative anti-diabetic remedies with better risk-benefit ratios and greater patient acceptability [1, 2, 4]. Plants have always been sources of drugs and many of the existing drugs have originated from plants directly or indirectly. Ethnobotanical studies have reported more than 1200 plant species with potential anti-diabetic effects [5].

The dried fruit of *Vaccinium arctostaphylos* L. (Caucasian whortleberry, Ericaceae family), hereafter referred to as whortleberry, is taken traditionally at the dose of 5 g per day to treat diabetes mellitus and hypertension in Iran, Turkey and Caucusus. The plant fruit is also used as a food in the above regions [6, 7, 8]. Whortleberry fruit is rich in bioactive anthocyanins and thus it can be regarded as a functional food [7]. Anthocyanins display a wide range of biological activities including anti-diabetic, anti-obesity, anti-hypertensive, cardioprotective, cataract preventing as well as microcirculation and vision improving activities [9-12]. Diabetes mellitus is commonly associated with obesity, hypertension, coronary heart disease, cataract and retinopathy [1-4]. Considering these data, whortleberry may have beneficial effects in the treatment of diabetes mellitus and its co-morbidities. Thus, the potential antihyperglycemic effect of whortleberry is worth investigation. Very little research has been conducted on the anti-hyperglycemic effect and safety of whortleberry. Alloxan-induced diabetes in the rat is an animal model of type 1 diabetes [13]. The ethanolic extract of whortleberry fruit reduced post-prandial blood glucose level in alloxan-diabetic Wistar rats [14]. Further, administration of the whortleberry leaf extract (250 mg every 8 h) to mildly hyperglycemic type 2 diabetic patients without any other anti-hyperglycemic agent lowered blood levels of fasting glucose significantly compared with baseline in a 4-week randomized, double-blind, placebo-controlled clinical trial. However, the effects on the glycosylated hemoglobin (HbA1c) level as a measure of glycemic control and blood insulin levels were not determined [8]. It should be noted that the effects of whortleberry on the blood levels of fasting glucose, HbA1c, insulin and liver/kidney function tests in the diabetic rats have not been studied so far. Further, as yet the safety and phytochemistry of the whortleberry growing in Iran have not been investigated. Thus, we tested the effects of whortleberry fruit and leaf extracts on the blood levels of fasting glucose, HbA1c, insulin, creatinine and the liver enzymes SGOT and SGPT in the model of alloxan-induced diabetes of rat. Moreover, a study to determine the LD_{50} (median lethal dose) of the extracts as a measure of acute

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toxicity was conducted in rats. The extracts were also standardized through determining the total anthocyanin content.

Materials and Methods

Whortleberry

Whortleberry was collected from the lands of the Ardebil province of Iran in October and its identity was authenticated by a botanist (Y. Ajanii). A voucher specimen of the plant (number 15062) was deposited in the Tehran University Central Herbarium. The fruits and leaves were separated from the plant, washed and dried in shade at room temperature. The dried fruits and leaves were ground into powder.

Preparation of the fruit and leaf extracts

The dried fruit/leaf powder (1000 g) was extracted with ethanol/water (70/30) as the solvent in a percolator three times, the solvent was completely removed from the hydroalcoholic extracts at 42 ºC by Rotavapor and 20 g and 25 g dried extracts of fruits and leaves were produced respectively [15].

Measurement of total anthocyanins

The total anthocyanin contents of the extracts were measured using the spectrophotometric method. 1 g of the extract was solved by 15:85 v/v of hydrochloric acid 1.5 N in ethanol 95%. The solution was filtered through paper filter into a 250 mL volumetric flask and diluted with solvent to 250 mL. The absorbance of the solution was measured at 535 nm wavelength. Measurements of total anthocyanins on the sample were replicated three times. The results were expressed in mg of total anthocyanins per 10 g of the extract [16]:

mg of total anthocyanins per 10 g of extract = (Ab × 25000)/98.2

Ab = Absorbtion of solution.

Drugs

Alloxan and glibenclamide (purity above 99%) were purchased from Sigma. For dilution, the extracts were dissolved in physiological saline and glibenclamide was dissolved in physiological saline by DMSO (dimethyl sulfoxide) (Merck) (10%). All drugs were prepared immediately before use.

Animals

Male adult Wistar rats (200 - 250 g) from our own breeding colony were used. Animals were maintained under standard environmental conditions and had free access to standard rodent feed and water.

Induction of diabetes

Animals were given alloxan in a single intraperitoneal injection at a dose of 125 mg/kg. Two weeks after the injection of alloxan, diabetic rats with fasting (after food deprivation for 8 h) blood glucose levels above 200 mg/dl were used for the experiments [13].

Experimental protocol

The animals were randomly divided into eleven groups (N=10 in each group):

Group I: Normal healthy control rats received physiological saline.

Group II: Normal healthy control rats received 10% DMSO in physiological saline (3 ml/kg).

Group III: Alloxan-diabetic rats received physiological saline.

Group IV: Alloxan-diabetic rats received 10% DMSO in physiological saline (3 ml/kg).

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Group V: Alloxan-diabetic rats received the fruit extract (250 mg/kg).

Group VI: Alloxan-diabetic rats received the fruit extract (500 mg/kg).

Group VII: Alloxan-diabetic rats received the fruit extract (1000 mg/kg).

Group VIII: Alloxan-diabetic rats received the leaf extract (250 mg/kg).

Group IX: Alloxan-diabetic rats received the leaf extract (500 mg/kg).

Group X: Alloxan-diabetic rats received the leaf extract (1000 mg/kg).

Group XI: Alloxan-diabetic rats received glibenclamide (5 mg/kg in 3 ml/kg DMSO 10%).

The extract and glibenclamide were administered at doses that when gavaged once, lowered the fasting blood glucose levels of the alloxan diabetic rats significantly compared with the control diabetic rats. Further, the data given here relate to the doses that not only did not cause any mortality in the diabetic rats after 8 weeks' daily gavage but also the effect of each dose on the blood glucose level at the endpoint was significantly different from the other dose of the same substance. Each animal was used only once in all experiments. Animals were gavaged once a day for 8 weeks. At the end of the eighth week, while the rats had been deprived from food for 8 h, blood was drawn from their tail veins and the blood glucose, HbA1c, insulin, creatinine and the liver enzymes SGOT and SGPT levels were measured.

Biochemical assays:

Fasting blood serum glucose level was measured by the glucose oxidase method (Pars Azmoon kit), blood glycosylated hemoglobin (HbA1c) level was measured by ion-exchange chromatography using DSS Pink-300 test kit (Drew Scientific Limited, UK) and the serum creatinine and SGOT and SGPT levels were determined using standard enzymatic kits produced by the Pars Azmoon company (Tehran, Iran) and an auto analyzer (Hitachi 902, Japan) from the whole blood immediately after drawing the blood sample. The serum insulin levels were measured by a sensitive rat insulin radioimmunoassay kit (Linco Research, Inc., St. Charles, MO) [17].

Determination of LD_{50}s of the fruit and leaf extracts

To calculate LD_{50} (median lethal dose), each extract was administered via gavage to groups of 30 healthy male and female Wistar rats at the doses of 50 mg/kg, 100 mg/kg, 0.5 g/kg, 1 g/kg, 5 g/kg, 10 g/kg and 15 g/kg once and the number of dead animals in each group within 72 hours was determined [18].

Statistical analyses

All data are expressed as mean ± standard error of the mean. One way ANOVA followed by Tukey's post hoc test was used for data analyses. $p<0.05$ was taken as statistically significant.

Results

Measurement of total anthocyanins

The total anthocyanin contents of the fruit and leaf extracts were 21.6395 and 7.3828 mg per 10 g of the extracts respectively.

Effects on the blood glucose levels:

There was no significant difference between the blood glucose levels of the normal control rats receiving physiological saline (group I) and the normal control rats receiving 10% DMSO in physiological saline (group II) after 8 weeks ($p > 0.05$). The blood glucose

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level of the diabetic control group receiving 10% DMSO in physiological saline (group IV) was not significantly different from the blood glucose level of the diabetic control group receiving physiological saline (group III) at the end of 8 weeks ($p > 0.05$). The blood glucose levels increased significantly in the diabetic control groups III and IV after 8 weeks of alloxan administration compared with the groups I and II ($p < 0.01$). The extracts and glibenclamide reduced the blood glucose levels of the diabetic rats significantly compared with the diabetic control groups after 8 weeks of administration (Table 1).

Effects on the blood HbA1c levels:

The blood HbA1c level of the normal control group receiving physiological saline (group I) was not significantly different from the blood HbA1c level of the normal control group receiving 10% DMSO in physiological saline (group II) after 8 weeks ($p > 0.05$). There was no significant difference between the blood HbA1c level of the diabetic control group receiving 10% DMSO in physiological saline (group IV) and the blood HbA1c level of diabetic control group receiving physiological saline (group III) at the end of 8 weeks ($p > 0.05$). However the blood HbA1c levels in the diabetic control groups (groups III and IV) were significantly higher than the blood HgA1c levels of the normal control groups (groups I and II) ($p < 0.01$). The extracts and glibenclamide reduced significantly the blood HbA1c levels of the diabetic rats compared with the control diabetic groups after 8 weeks ($p < 0.01$) (Table 1).

Table 1- Effects of the whortleberry fruit and leaf extracts on the fasting blood glucose, glycosylated hemoglobin (HbA1c) and insulin levels after daily gavage for eight weeks in rats

Treatment groups (N = 10 in each group)	Blood glucose (mg/dl)	Blood HbA1c (%)	Blood insulin (µ U/ml)
Normal control (physiological saline)	102.52 ± 3.42	7.22 ± 0.13	15.47 ± 0.73
Normal control (10% DMSO in physiological saline)	100.04 ± 2.78	7.24 ± 0.16	15.62 ± 0.44
Diabetic control (physiological saline)	357.23 ± 2.61*	12.13 ± 0.21*	6.03 ± 0.14*
Diabetic control (10 % DMSO in physiological saline)	359.87 ± 3.67*	12.17 ± 0.18*	6.24 ± 0.26*
Fruit extract (250 mg/kg)	219.2 ± 5.21**	8.9 ± 0.13**	12.74 ± 0.16**
Fruit extract (500 mg/kg)	117.3 ± 4.23**	6.7 ± 0.04**	13.03 ± 0.21**
Fruit extract (1000 mg/kg)	109.14 ± 6.12**	6. 2 ± 0.23**	16.81 ± 0.26**
Leaf extract (250 mg/kg)	238.72 ± 5.42**	9.61 ± 0.13**	12.17 ± 0.92**
Leaf extract (500 mg/kg)	138.12 ± 3.14**	8.14 ± 0.22**	14.11 ± 0.14**
Leaf extract (1000 mg/kg)	126.71 ± 4.52***	7.8 ± 0.01**	15.01 ± 0.09**
glibenclamide (5 mg/kg in 3 ml/kg DMSO 10%)	105.12 ± 2.71**	5.74 ± 0.03**	15.71 ± 0.80**

The data are given as mean ± standard error of the mean.
*p<0.01, compared with normal healthy rats.
**p<0.01, compared with diabetic control rats.
***p<0.05, compared with diabetic control rats.

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Effects on the blood insulin levels

No significant difference was observed between the blood insulin levels of the normal control rats receiving physiological saline (group I) and the normal control rats receiving 10% DMSO in physiological saline (group II) after 8 weeks ($p > 0.05$). There was no significant difference between the blood insulin levels of the diabetic control group receiving 10% DMSO in physiological saline (group IV) and the diabetic control group receiving physiological saline (group III) at the end of 8 weeks ($p > 0.05$). The blood insulin levels of the diabetic control groups III and IV decreased significantly after 8 weeks of alloxan administration compared with the groups I and II ($p < 0.01$). The extracts and glibenclamide increased the blood insulin levels of the diabetic rats significantly compared with the diabetic control groups after 8 weeks of administration (Table 1).

Effects on the blood SGOT, SGPT and creatinine levels:

The extracts and glibenclamide did not have any significant effects on the blood SGOT, SGPT and creatinine levels in the diabetic rats compared with the diabetic control groups after 8 weeks of administration.

Determination of LD_{50}s of the fruit and leaf extracts

The LD_{50}s of the extracts were more than 15 g/kg.

Discussion

The results indicate that the total anthocyanin content of the Iranian whortleberry fruit is about three times greater than the leaf anthocyanin content. The anthocyanin content of the Iranian whortleberry fruit is also comparable to the anthocyanin content of the Turkish whortleberry fruit (1420 mg/100 g dry fruit weight) [7]. Moreover, whortleberry leaves and fruits reduce the blood glucose and HbA1c levels but increase the blood insulin levels significantly without causing any hepatic and renal dysfunctions in the alloxan-induced diabetic rats. The LD_{50}s of the extracts show that whortleberry fruits and leaves are relatively nontoxic. The results are also in line with the previous studies regarding the anthocyanin content of whortleberry [7] and the anti-hyperglycemic effects of whortleberry in rats [8] and type 2 diabetic patients [14].

Considering that HbA1c indicates glycemic control during the past 2 to 3 months in the diabetic patients [19], the present study demonstrates that the whortleberry fruit and leaf extracts may effectively control glycemia in the alloxan-induced diabetic rats without hepatic and renal toxicities.

The results indicate that the mechanism of anti-hyperglycemic effect of whortleberry may at least partly be through increasing the blood insulin level. The active constituents involved in the effects of the extracts were not determined in the present study. Thus, the active constituents need to be identified. However, anthocyanins may be responsible for the effects of the extracts because published data suggest that anthocyanins may decrease blood glucose by improving insulin resistance, protecting β cells, increasing secretion of insulin and reducing digestion of sugars in the small intestine. The mechanisms of action are primarily related to their antioxidant properties, but enzymatic inhibition and other pathways may also be relevant [20]. Apart from anthocyanins, whortleberry also contains

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chlorogenic acid [21] and myricetin [8] which may be responsible for the anti-hyperglycemic effect through various mechanisms [8, 22-24]. Chlorogenic acid may lower blood glucose by inhibiting its synthesis in the liver and by reduction of dietary glucose absorption in the intestines [23]. Further, myricetin may reduce blood glucose by stimulating glucose utilization as well as by inhibiting the dietary glucose absorption [24].

Conclusion

In conclusion, further studies into the effects of whortleberry and their mechanisms in the other animal models of diabetes and conduction of clinical trials on the efficacy and safety of whortleberry fruits and leaves in the treatment of type 1 and type 2 diabetic patients are recommended.

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